CHEMICAL PROTECTION OF MEG AGAINST IONIZING RADIATION
REPORT I THE PROTECTIVE EFFECT OF MEG AGAINST DEATH DUE TO X-RAY IRRADIATION IN MICE

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放射線に対する MEG の化学的保護に関する研究
第I報 マウスのX線致死に対する保護について

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致死線量X線照射マウス（ddN 均一系，8週令）における AET_{Br}，MEG_{Br}（AET_{Br}の中性溶液），MEG_{SO4} の生存率に対する保護効果を調べ次の結果を得た．

MEG_{Br} の最有効投与量（至適投与量）は250 mg/kgであつた．この量を投与したマウスの30日生存率は90～100%であつた．最有効投与時間（至適投与時間）は照射前15分ないし10分であつたが，直前投与でも著明な保護効果を示した．

AET_{Br} は照射前10分よりも30分投与の方がより有効であつた．

MEG_{SO4} は MEG_{Br} と比較して，モル濃度からみれば放射線致死に対する効果および毒性においてほゞ等しかつた．しかしpHの調整が不必要である点が便利である．

雄と雌に対する保護効果には顕著な差はなかつた．

保護効果は照射時間の延長につれて除々に減少した．

種々のMEG投与量，投与時間のすべての群を集計した保護マウスの死亡は，照射後12日目に最大の頻度を持ち，非保護マウスのそれは照射後6日目と12日目の二つの極大値を示した．

Introduction

Among the large number of chemical substances which have protective action against radiation injuries, AET_{Br} (s, β-aminoethylisothiuronium-Br. HBr) or MEG (β-mercaptoethylguanidine)reported by Doherty et al[1], has excellent properties that it possesses high protective activity, relative low toxicity and low sensitivity for atmospheric oxygen as compared with other protectors. Other investigators have reported that AET has a protective action against X-ray irradiation in vivo as well as in vitro. Although the use of these compounds in man

is hazardous because of the toxic effects it produces at relatively low dose, clinical applications of protective agents have been attempted as they protect normal tissues in radiation therapy of cancer, etc[2]. Other sulfhydryl compounds, such as cysteamine, cysteine[3] and MPG[4], are effective in providing protection against radiation injuries. However, the mechanism of radiation protection, that is to say, the manner in which chemical compounds demonstrate their protective action, is a subject of considerable challenge. Among the many hypotheses, the following seems to be the likely mechanism. First, it competes with the biological molecules against the free radicals produced by irradiation of water. Second, it reacts with sensitive biological molecules through either covalent bond formation or binding of the enzyme-substrate type to make them more resistant to radical attack. Third, it lowers the oxygen tension and reduces radiosensitivity. Forth, it stimulates process necessary for recovery from radiation damage and thus bring about a more rapid recovery. Finally, it temporarily forms mixed disulfide with sulfhydryl groups of protein and protects the protein.

In biological systems simpler than whole animals, experimental evidence has been obtained to support each hypothesis[5][6][7][8].

In order to elucidate the protective mechanism of MEG or other protective agents,a study was made on the protective effects of MEG against the lethal effect of X-ray and neutron irradiation, hematopoietic and intestinal injuries, and radiation degradation of chemical substances, and on the distribution of MEG in various organs.

This paper describes the protective effect of MEG against death due to X-ray irradiation in mice.

Material and method

The radiation source used was a Toshiba KXC-18-2 with tube voltage, 180 kVp; filter, 0.5 mm Cu+0.5 mmAl; HVL, 1.18 mmCu and target to mice distance, 65 cm. Tube current was 25 mA except for the study of the effect of irradiation time. Dose measurements were made with a Victoreen Radocon 575 (probe 601) placed in the center of one of acrylite irradiation boxes. All mice were exposed to 800 r of total body X-ray irradiation at about 50 r/min. This dose is lethal for unprotected mice.

In all experiments, mice (ddN uniform strain) were 8 weeks old with body weight of 23 ±2g (female) and 25±2 g (male) when irradiation was begun. After irradiation, mice were housed in metal cages (4 or 5 animals per cage) and were provide a free supply of CLEA pellet and water.

Two percent AET_{Br} (supplied by Takeda Phamaceutical Co., and prepared by the author) was adjusted at pH 7.0 with dilute NaOH solution and used immediately after preparation. In some experiments, MEG_{SO4} and unadjusted AET_{Br} were used. These protective agents were administered by intraperitoneal injection.

By neutralization, AET transforms into MEG through intermediate compound. MEG once formed is stable and does not at any pH transfrom back to AET. As transformation of AET into MEG is not quantitative and other compounds are produced, administered doses of MEG were shown as doses of AET.

MEG_{SO4} was prepared by Taguchi to reduce the toxicity of bromine ion[9].

Experiments were carried out on the following subjects: I) The most effective dose of MEG_{Br}; II) The most effective time of administration of MEG_{Br}; III) The protective activity of AET_{Br}; IV) The effect of sex on protective activity; V) The protective activity of MEG_{SO4}; VI) The effect of irradiation time on protective activity.

In the estimation of protective activity the 30-day survival rate was employed.

Results and discussion

I) The most effective dose of MEG_{Br}

It has been already reported that the most effective dose of AET or MEG varies according to the strain of mice[10)11)12)]. Furthermore, it is also related to irradiation dose as described in Report II. The protective effects in administration of graded levels of MEG were determined by the survival rate of mice. Table I shows the relationship between the dose of MEG administered and the survival rate of mice exposed to 800 r of total body irradiation. In all tables except Tables III and V, milligram of MEG administered per kilogram of body weight is not actual MEG dose but the dose of AET. Untreated mice exposed to X-ray was used as control.

Table I The relationship between MEG dose administered and survival rate (administration time: 10 minutes before irradiation, ♀)

MEG (mg/kg)	Survival(%)	No. of mice
Unprotected	4	50
150	40	10
200	80	10
250	96	50
350	70	20

As shown in Table I, MEG had a marked effect in protecting mice exposed to nearly lethal dose from dying. Particularly, MEG showed the highest activity when the administration dose was 250 mg/kg. Below and above 250 mg/kg, the activity gradually decreased. When the dose exceeded 400 mg/kg, some mice died immediately or during irradiation due to the toxicity of MEG.

The first death in unprotected mice occured on the fifth to sixth day after irradiation and almost mice died within 20 days. In protected groups, mice began to die on about the tenth day after irradiation and death after the 20th day was rarely observed.

A example of daily mortality after irradiation with and without protection is shown in Fig. 1. Calculation of daily mortality was carried out in mice protected with various doses of MEG and various administration time. As seen in Fig. 1, in any administered dose and time MEG significantly modified deaths due to intestinal disorders as seen in deaths on the fifth to sixth day after irradiation. However it cannot be concluded that MEG was more effective for intestinal disorders than for hematopoietic disorders.

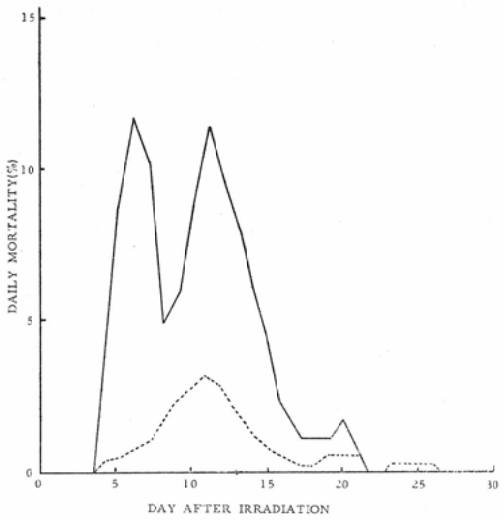

Fig. 1 The daily mortality after exposure to X-ray (daily mortality was calculated as a 3-day running average by dividing the number of mice dying during a day by the total number of irradiated mice) —— Unprotected, ····· Protected

II) The most effective time of administration

Table II shows the relationship between administration time and survival rate. Good protection was shown in three groups injected with MEG immediately, 10 minutes and 30 minutes before irradiation. Particularly, the first two groups showed more than 90 % survival rate. Although there was no significant difference in the 30-day survival rate between the two groups, according to the data on body weight loss and the 60-day survival rate, MEG administered 10 miuntes before irradiation was found to be more effective than MEG administered immediately before irradiation. In the latter group, 5～10 % of the mice surviving more than 30 days did not recover body weight loss and almost all the mice which did not recover weight loss died the following month. In the former group, most of the mice recovered body weight loss and only few mice died the following month.

Table II The relationship between administration time and survival rate
(MEG: 250㎎/㎏)

Administration time	Survival (%)	No. of mice
60 minutes before irradiation	15	20
30 〃	70	20
10 〃	100	10
Immediately befor irradiation	90	10
Immediately after irradiation	0	10
30 minutes 〃	0	10

When administered immediately and 30 minutes after irradiation, MEG not only failed to increase the survival rate compared to that of unprotected mice but slightly decreased the mean survival time.

III) Protective activity of AET_{Br}

Table III shows the protective activity of AET_{Br} unadjusted to neutral pH. AET when administered 30 minutes before irradiation was more effective than that administered 10 minutes before irradiation. This indicates that AET needs 20 to 30 minutes after injection to change into effective MEG in view of the finding that MEG was most effective when administered 10 minutes before irradiation.

When using AET for the study of protective effect, it therefore must be confirmed whether sulfhydryl radical is free and transguanylation has occured. In this study, Nitropurruside and Sakaguchi reactions were used in free SH radical test[13] and monosubstituent of guanidine test[14], respectively.

Table III The protective activity of unadjusted AET_{Br}
(AET solution : 2%, pH 4.5)

AET mg/kg	Administration time	Survival (%)	No. of mice
250	60 minutes before irradiation	17	12
	30 〃	80	12
	10 〃	58	12
300	30 〃	71	12
	10 〃	50	12

IV) Effect of sex on protective activity

In general, radiosensitivity of male is slightly higher than that of female, but no marked difference was found in the 30-day mortality of protected male and female mice. The results are shown in Table IV. The 30-day survival rate was 92 % and 90 % for protected male and female mice, respectively, while it was 0 % for unprotected male and female mice.

Table IV The effect of sex on the protective activity
(MEG : 250mg/kg, administration time : 10 minutes before irradiation)

Sex	Treatment	Survival (%)	No. of mice
female	unprotected	0	10
	protected	90	10
male	unprotected	0	17
	protected	92	21

V) Protective activity of MEG_{SO_4}

In order to reduce the toxicity attributable to bromine by substituting bromine ion to sulfate ion and increase the protective activity, MEG_{SO_4} was first synthesized by Taguchi.

However, MEG_{SO_4} was almost equally effective and toxic as MEG_{Br}.

There was no significant difference in the effectiveness of MEG_{SO_4} solution with and without the adjustment of pH when compared with the result obtained with AET_{Br}. This result agrees with the fact that MEG_{SO_4} exists in an effective form, namely, free SH form in unadjusted solution (see Table V).

Table V The protective activity of MEG SO_4 (MEG SO_4 : 160mg/kg)

pH	Administration time	Survival (%)	No. of mice
5.6 (non-adjsted)	10 minutes before irradiation	80	10
	30 〃	70	10
7.0 (adjusted)	10 〃	80	10
	30 〃	90	10

VI) Effect of irradiation time on protective activity

As seen in section (II), the protective activity of MEG varied with administration time. For the same reason it is felt that activity is affected by irradiation time. To determine the effect of irradiation time, eight groups of mice obtained by the combination of four irradiation times and two administration times were used. The irradiation time was 15, 30, 45 and 60 minutes, in which the dose rate was 53, 37, 18 and 13 r/min, respectively. The administration time was 10 minutes before irradiation and immediately before irradiation.

The 30-day survival rate and recovery rate of body weight are shown in Tables VI and VII. As seen in Table VI, MEG had the highest activity for the group administered 10 minutes before irradiation and irradiated for 15 minutes. In general, protective activity decreased with the elongation of irradiation time.

The recovery rate of body weight in 30 day survivors fell with the elongation of irradiation time.

Table VI The effect of the irradiation time on the protective activity of MEG (MEG : 250mg/kg)

Administration time	Dose rate (r/min)	Irradiation time (min)	Survival (%)	No. of mice
Unprotected	53	15	0	10
	18	45	0	10
Immediately before irradiation	53	15	95	20
	27	30	90	20
	18	45	80	20
	13	60	85	20
10 minutes before irradiation	53	15	100	10
	27	30	80	10
	18	45	70	10
	13	60	70	10

Table Ⅶ The effect of irradiation time on the protective activity of MEG in the recovery of the body weight

Administration time	Irradiation time (min)	Body weight (g)		Recovery rate (%)	No. of mice showing no weight recovery
		Before irradiation	30th day after irradiation		
Immediately before irradiation	15	23.5	24.0	102	3 (19)
	30	23.8	24.3	102	4 (18)
	45	23.9	22.1	92.0	8 (16)
	60	23.3	20.6	88.4	15 (17)
10 minutes before irradiation	15	23.6	24.2	103	1 (10)
	30	24.0	24.2	101	1 (8)
	45	23.8	23.7	99.5	1 (7)
	60	23.8	21.4	89.9	5 (7)

However, all groups has a survival rate greater than 70 %. In spite of irradiation time extending over 60 minutes, survival rate was 85 % for those administered immediately before irradiaition and 70 % for those administered 10 minutes before irradiation. These values were higher than those expected from the data on survival for those administered 60 minutes before irradiation and irradiated for 15 minutes (15 % survival, see Table II).

To confirm the significance of the apparent difference of these two groups, the 30-day survival was compared between two groups having the same time from administration of MEG to the completion of irradiation. For one group, the irradiation time was 60 minutes and administration of MEG was 15 minutes before irradiation and for the other, they were 15 minutes and 60 minutes, respectively.

The survival rate of the former was 30 % (9/30) and that of the latter was 73 % (22/30). The difference in protective activity was markedly significant. Although data are not available at present stage for quantitative evaluation, the effective time of MEG in body may be roughly estimated from these results. Further, the accumulation of MEG in various organs and the chemical changes in body after injection of S^{35}-labeled MEG will be studied to determine the effective time.

The protective activity of MEG in brief irradiation (about 1 minutes) will be reported elsewhere.

Summary

The protective effects of AET_{Br}, MEG_{Br} and MEG_{SO_4} were determined by the survival rate of mice (ddN uniform strain, 8 weeks old) exposed to 800 r of total body X-ray irradiation. This dose is lethal for unprotected mice and the following results were obtained.

1) The most effective dose (optimum dose) of MEG_{Br} was 250 mg/kg. The 30-day survival rate of mice injected that dose was 90 to 100 %.

2) The most effective time of administration (optimum time) was 10 minutes before irradiation.

3) AET_{Br} was more effective when administered 30 minutes before irradiation than 10 minutes before irradiation, but slightly less effective than MEG_{Br}.

4) MEG_{SO4} was almost equal to MEG_{Br} in effectiveness against radiation mortality and the toxicity on molar basis but it is convenient in that abjustment of of pH is unnecessary.

5) There was no significant difference in protection activity between male and female mice.

6) The protective activity decreased with elongation of irradiation time.

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References

1) D.G. Doherty and W.J. Burnett, Jr.: Proc. Soc. Exptl. Biol. Med., 89, 312 (1955).
2) W.N.C. Brown: Brit. J. Radiol., 28, 325 (1955).
3) Z.M. Bacq: Acta Radiologica, 41, 47 (1954).
4) D.G. Doherty, R. Shapira and W.J. Burnett, Jr.: Radiation Research, 7, 22 (1957).
5) L. Eldjarn and A. Phill: Mechanisms in Radiobiology, II, 231 (1960).
6) H.I. Kohen and S.E. Gunter: Radiatation Research, 13, 250 (1960).
7) C.V. Heer, D.W. Bekkum and J.A. Cohen: PUAE (2nd) 23, 42 (1958).
8) M.A. Gerebtzofe and Z.M. Bacq: Experientia, 10, 34 (1954).
9) T. Taguchi: in press (1960).
10) Y. Katsuhara: Nipp. Acta. Radiol., 19, 73 (1959).
11) T. Muta and S. Koiwa: Nipp. Acta. Radiol., 19, 1569 (1959).
12) T. Hashimoto: Nipp. Acta. Radiol., 20, 346 (1960).
13) R.R. Grunter and P.H. Phillips: J. Biol. Chem., 181, 820 (1949).
14) C.J. Weber: J. Biol. Chem., 86, 217 (1930).